

The first of these limitations is electroosmosis, a simultaneous movement of the separation medium due to the presence of charges on the walls of the capillary or of the channel. As this movement is often variable over time and nonuniform, it hampers the reproducibility of the measurements and the resolution. Numerous methods have been proposed for combating it, such as treating the surface of the capillaries by adsorption of essentially neutral species on the walls of the separating channel prior to the actual separation (Wiktorowicz et al., Electrophoresis, 11, 769, 1990, Tsuji et al., J. Chromatogr. 594, 317 (1992), or by treating the capillary with an acidic solution (Fung et al., Anal. Chem. 67, 1913, (1995)). These methods have the advantage of being inexpensive and of being capable of being repeated several times in order to regenerate a capillary, but often only partially reduce the electroosmosis. Methods for the irreversible grafting of an essentially neutral polymeric layer on the walls have also been proposed, as for example described in US 4 680 201. Ready-to-use treated capillaries are thus commercially available. These irreversibly treated capillaries lead to a good reduction of electroosmosis for a certain number of separations, but their shelf life is limited and their cost is high.

Another major disadvantage of electrokinetic separations in polymer solutions is that the resolution and the separable range of sizes are better with solutions which are relatively concentrated and of high molecular masses (Mitnik et al., J. Chrom. A, 710, 309 (1995); Goetzinger et al., Electrophoresis, 19, 242, 1998)). This is attributed to deformations of the separating matrix which limits the resolution for large size analytes and which are greater the lower the molecular mass of the matrix and the lower its concentration. On the other hand, the viscosity of a polymer solution increases very rapidly when the

molecular mass and the concentration are increased. The application of entangled water-soluble polymer solutions is therefore limited by the very great difficulty, and as a last resort the impossibility, of  
5 introducing into a small size capillary (typically less than 100 micrometres) a solution of very high viscosity. Finally, it should be noted that the separation range accessible to capillary electrophoresis may be extended to the largest sizes by  
10 the use of pulsed fields. Phenomena of DNA aggregation are encountered in this case which limit the scope of the improvement, and which are also greater, the lower the viscosity of the medium.

15 To solve the dilemma posed by the search for a low viscosity for injecting the separation medium into the channel, and for resistant topological obstacles for the separation, which in fact lead to a high viscosity, some authors have proposed using a polymer medium whose  
20 viscosity decreases considerably during a rise in temperature. This type of medium has the advantage of allowing the injection of the said medium into the capillary at high temperature in a low viscosity state, and separation at a lower temperature in a higher  
25 viscosity state exhibiting good separation performance features, as is commonly carried out in gel electrophoresis, in particular with agarose.

In applications WO 94/10561 and WO 95/30782, media are  
30 proposed in particular which allow easier injection by raising the temperature. Essentially described in these patent applications are microgels capable of decreasing in volume at high temperature (thus leading to a dilute solution of discontinuous particles of low viscosity)  
35 and of swelling at low temperature to the extent of fully occupying the separating channel (thus conferring a gelled character and good separation properties on the medium).

However, these separation media have a viscosity which decreases more or less rapidly with temperature: it is therefore necessary to introduce them into the capillary at a temperature greater than the temperature at which the separation occurs, which may have various disadvantages. On the one hand, in capillary electrophoresis apparatus, it is very difficult to thermostat the entire capillary, and it is therefore difficult to use in an automated fashion a polymer which would only be injectable at a temperature which is markedly greater than room temperature. It would be possible to envisage a solution which is not very viscous at room temperature, and which has a high viscosity and good separation properties at a lower temperature, but that involves carrying out the separations at low temperatures, which is not possible for all analytes. In particular, it is known that for DNA sequencing, an optimum resolution of the "compressions" is obtained at a relatively high temperature (of the order of 50-60°C), which is incompatible with the preceding principle.

Application WO 98/10274 proposes, for its part, a molecular separation medium comprising at least one type of block copolymer which is in solution at a first temperature and in a gel-type state at a second temperature. This medium comprises, in addition, a buffer whose role is to dissolve the block copolymer at a first temperature, and to cause it to transit towards the gel state at the said second temperature without interrupting the separation process, and without preventing a return to a soluble state during the return to the said first temperature. More specifically, the polymers described are triblock polymers of low molecular masses (typically less than 20,000), of the polyoxyethylene-polyoxypropylene-polyoxyethylene (POE-POP-POE) family and more specifically still (POE<sub>99</sub>-POP<sub>69</sub>-POE<sub>99</sub>, where the subscripts represent the numbers of monomers of each